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Sugar utilization patterns and respiro-fermentative metabolism in the baker's yeast *Torulaspora delbrueckii*

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The highly osmo- and cryotolerant yeast species *Torulaspora delbrueckii* is an important case study among the non-*Saccharomyces* yeast species. The strain *T. delbrueckii* PYCC 5321, isolated from traditional corn and rye bread dough in northern Portugal, is considered particularly interesting for the baking industry. This paper reports the sugar utilization patterns of this strain, using media with glucose, maltose and sucrose, alone or in mixtures. Kinetics of growth, biomass and ethanol yields, fermentation and respiration rates, hydrolase activities and sugar uptake rates were used to infer the potential applied relevance of this yeast in comparison to a conventional baker's strain of *Saccharomyces cerevisiae*. The results showed that both maltase and maltose transport in *T. delbrueckii* were subject to glucose repression and maltose induction, whereas invertase was subject to glucose control but not dependent on sucrose induction. A comparative analysis of specific sugar consumption rates and transport capacities suggests that the transport step limits both glucose and maltose metabolism. Specific rates of CO_2 production and O_2 consumption showed a significantly higher contribution of respiration to the overall metabolism in *T. delbrueckii* than in *S. cerevisiae*. This was reflected in the biomass yields from batch cultures and could represent an asset for the large-scale production of the former species. This work contributes to a better understanding of the physiology of a non-conventional yeast species, with a view to the full exploitation of *T. delbrueckii* by the baking industry.

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INTRODUCTION

Torulaspora delbrueckii and *Saccharomyces cerevisiae* are major constituents of the yeast flora present in corn and rye bread doughs (Hahn & Kawai, 1990; Almeida & Pais, 1996a). The biotechnological interest in *T. delbrueckii* has increased in recent years due to its particularly high freezing and osmotic tolerance (Almeida & Pais, 1996b; Ok & Hashinaga, 1997; Hernandez-Lopez *et al.*, 2003; Alves-Araújo *et al.*, 2004a). Moreover, strains of *T. delbrueckii* have been shown to display dough-raising capacities similar to those of commercial baker's yeasts (Almeida & Pais, 1996b), thus reinforcing their potential application in the baking industry. However, few reports exist on the genetics, biochemistry and physiology of *T. delbrueckii*, in contrast to the vast knowledge on the traditional baker's yeast *S. cerevisiae*.

Two main aspects must be considered when selecting a yeast strain for the baking industry (Benitez *et al.*, 1996): effective biomass production in molasses, and dough-leavening ability. Sucrose is the primary carbon and energy source for growth in molasses, the industrial substrate used for large-scale baker's yeast production. Expression of invertase, the hydrolytic enzyme required to convert sucrose into glucose and fructose, is repressed by high glucose concentrations (Mormeneo & Sentandreu, 1982). Despite the high levels of invertase activity required for growth in molasses, there is evidence that the capacity of *S. cerevisiae* to ferment high sucrose concentrations, like those present in sweet bread doughs, is inversely related to the activity of this enzyme (Attfield & Kletsas, 2000). This is usually ascribed to the reduction in water activity resulting from sucrose hydrolysis and the consequent negative effect on yeast performance. Although there is a small amount of free sugars in the flour (0.3–0.5 % – essentially glucose, fructose,

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sucrose and maltose), the maltose gradually released from starch as a result of amylolytic activity represents the major fermentable sugar in the dough (Ponte & Reed, 1982). Maltose metabolism requires the presence of both a maltose transporter and a maltase. In *S. cerevisiae*, the constitutive internal maltase is considered sufficient to hydrolyse maltose, and sugar utilization is limited by maltose uptake (Goldenthal *et al.*, 1987). The efficiency in gas production is determined by high maltase and maltose transport activities (Higgins *et al.*, 1999).

The specific growth rate is a key control parameter in the industrial production of baker's yeast (van Hoek *et al.*, 1998). The biomass productivity of *S. cerevisiae* is limited by the aerobic fermentation occurring in high-sugar media (Crabtree effect), demanding a high-oxygen fed-batch cultivation method to keep the sugar concentration low and avoid fermentative metabolism. Redirection of the respiro-fermentative flux at high sugar concentrations, and consequent improvements in biomass yields, have been successfully achieved by alleviating glucose repression, either by overexpressing a protein involved in the repressing pathway (Blom *et al.*, 2000) or by engineering glucose uptake rates (Otterstedt *et al.*, 2004). Notably, glucose transport has been shown by different authors to play a fundamental role in the fate of glycolytic flux in *S. cerevisiae* (Diderich *et al.*, 1999; Ye *et al.*, 1999).

In glucose-limited oxygen-sufficient chemostat cultures, *T. delbrueckii* shows biomass yields similar to those obtained for *S. cerevisiae* and consistent with fully respiratory growth. As the oxygen feed rate decreases, *S. cerevisiae* is the first to switch to a respiro-fermentative metabolism, already showing a decrease in biomass yield at oxygen tensions still able to sustain full respiration in *T. delbrueckii*. However, *T. delbrueckii* shows considerably poorer growth than *S. cerevisiae* under strict anaerobic conditions (Visser *et al.*, 1990; Hanl *et al.*, 2005).

We have undertaken physiological and biochemical studies of *T. delbrueckii* in batch cultures with sugars present in molasses and in bread dough, using them alone and in mixtures. A strain isolated from traditional corn and rye bread dough in northern Portugal and showing particularly promising characteristics, *T. delbrueckii* PYCC 5321, was used. The resulting information on sugar utilization patterns, maltase and invertase activities, sugar uptake rates and respiration/fermentation rates contributes to a better evaluation of the potential offered by this yeast to the baking industry.

METHODS

Micro-organisms and growth conditions. The yeast strains used in this study were *Torulaspora delbruekii* PYCC 5321, isolated from homemade corn and rye bread dough in northern Portugal, and *Saccharomyces cerevisiae* PYCC 5325, isolated from commercial compressed baker's yeast. They are both deposited at the Portuguese Yeast Culture Collection, Caparica, Portugal. Stock cultures were maintained at 4 °C on slants of YPDA medium containing, per litre, 20 g glucose, 10 g peptone, 5 g yeast extract and 20 g agar.

Since sucrose is the primary carbon and energy substrate present in beet or cane molasses used for industrial baker's yeast production, the inoculum for all experiments was prepared in YPS medium, containing, per litre, 20 g sucrose, 40 g peptone, 20 g yeast extract, 2 g KH_2PO_4 and 1 g $MgSO_4.7H_2O$. Peptone (0118-17) and yeast extract (0127-17) were from Difco and sucrose from Merck. Cells were harvested from a 24 h culture, washed and used as inoculum.

Cultures were carried out in the same YP medium as used to prepare the inoculum but containing 20 g l^{-1} of sucrose, glucose, maltose or pairwise mixtures of these sugars. When indicated, yeasts were grown in a mineral medium (van Uden, 1967) supplemented with 20 g l^{-1} glucose, sucrose or maltose, with agitation in an orbital shaker (160 r.p.m.) at 30 °C. Growth was followed by measuring the OD_{640} of the culture. At specified times during exponential growth, biomass dry weight was also determined.

Analytical procedures. To determine sugar and ethanol concentrations in the growth medium, the cultures were sampled and immediately centrifuged at 16 000 ***g*** for 3 min. The supernatant was frozen and kept at −20 °C until analysed. Quantitative analysis of sugar and ethanol was based on HPLC, using a Gilson chromatograph equipped with a 132-RI detector and a Hypersil-SS-100, H^+ column at 30 °C with a 5 mM H_2SO_4 solution as the mobile phase at a flow rate of 0.45 ml min^{-1}. Due to poor resolution of sucrose and maltose in this system, these two sugars when used in a mixture were determined enzymically using the sucrose/D-glucose UV colorimetric method (Roche, 139041; Boehringer Mannheim) and the enzyme α-glucosidase (Roche, 124036).

Biomass yields were determined from the slopes of plots of biomass dry weight versus consumed sugar during exponential growth. The ethanol yield was determined by dividing the maximum ethanol concentration obtained by the consumed sugar and expressed as g ethanol per g substrate carbon. Each specific sugar consumption rate (q_{sugar}) was determined by dividing the specific growth rate (μ) by the biomass yield (Y_x) during exponential growth on the respective sugar.

Enzyme assays. To obtain cell-free extracts for the determination of enzymic activities, 25–30 mg of cell mass (wet weight) was collected at different times during growth, sedimented by centrifugation, and washed twice with cold homogenization buffer (0.1 M potassium phosphate buffer, pH 6.5). The pellet was resuspended in 0.45 ml of the same buffer and transferred into a tube containing 0.5 ml acid-treated glass beads (0.5 mm diameter). The mixture was vortexed for four periods of 0.5 min, separated by 1 min intervals on ice, centrifuged for 5 min at 1000 *g* (4 °C) and the supernatant used immediately for analysis. The total protein content in the cell-free extract was determined with the Bio-Rad protein assay kit based on the Bradford method (Bradford, 1976), and using bovine serum albumin as standard. Maltase activity was determined in the crude extract as described by Okada & Halvorson (1964) using *p*-nitrophenyl α-D-glucopyranoside (pNPG) as substrate. One unit (U) is defined as the amount of enzyme that produces 1 μmol *p*-nitrophenol in 1 min under the assay conditions. Invertase activity was assayed as described by Niederacher & Entian (1987) and is expressed as μmol glucose released from sucrose in 1 min per mg protein (U mg^{-1}).

Maltose and glucose transport. For sugar transport assays, the cultures were sampled at the indicated times and cells were harvested by centrifugation, washed twice with ice-cold water, suspended in water to a density of 35–45 mg dry weight of cells ml^{-1} and kept on ice. Zero-trans influx of labelled maltose or glucose

(Amersham) was determined at 30 °C. Ten microlitres of cell suspension was mixed with 30 µl 0.1 M potassium phosphate buffer (pH 5.0). The cell suspension was allowed to reach the temperature of the assay and the reaction started by adding 10 µl of an aqueous solution of [U-^{14}C]maltose (specific activity 610 mCi mmol^{-1}; 22.6 GBq mmol^{-1}) or [U-^{14}C]glucose (specific activity 310 mCi mmol^{-1}; 11.5 GBq mmol^{-1}) at the desired concentrations. After incubation for 5 s, 4.5 ml chilled water was added and the mixture immediately filtered through glass fibre filters (GF/C filters, Whatman). The cells on the filter were washed with 15 ml chilled water, the filter immersed in 5 ml scintillation liquid OptiPhase HiSafe II (LKB Scintillation Products) and the radioactivity measured using a Packard Tri-Carb 2200 CA liquid scintillation counter (Packard Instrument Co.), with correction for disintegrations per minute. Non-specific binding of radiolabelled sugar to the yeast cells and filter was determined in parallel by pouring ice-cold water immediately before the addition of the labelled sugar. For each sugar concentration, the reaction was performed in triplicate.

Fermentation and respiration rates. Fermentation and respiration rates were determined using the standard Warburg method (Umbreit *et al.*, 1964). Yeast strains were grown on YP medium supplemented with 20 g l^{-1} glucose, sucrose or maltose. Cells were harvested at the exponential growth phase (OD_{640} 0.8–0.9), washed twice with water and suspended in cold water to a cell density 10-fold higher than the original culture. This suspension was diluted in 0.1 M KH_2PO_4 buffer, pH 5.0, to a cell concentration allowing measurements of CO_2 production and O_2 consumption in the manometer of the Warburg apparatus during a period of approximately 60 min. The experiments were started by the addition of the sugar solution (final concentration 20 g l^{-1}) to the cell suspension and performed at 30 °C, in duplicate. Fermentation rates are expressed in mmol CO_2 produced per g dry weight of cells per hour, and respiration rates expressed in mmol O_2 consumed per g dry weight of cells per hour. The respiratory quotient (RQ) was calculated as the ratio between total CO_2 produced and the O_2 consumed.

RESULTS

Growth and sugar utilization patterns

To characterize growth and sugar utilization patterns of *T. delbrueckii* PYCC 5321, the yeast was cultivated in YP medium with glucose, sucrose and maltose, either as single carbon and energy source or in mixtures. The curves in Fig. 1 show the results obtained in sugar mixtures. In glucose-maltose (G-M) medium, maltose consumption became detectable only after glucose was no longer present (Fig. 1a). No diauxic growth curve was observed, i.e. there was no lag period preceding the utilization of the second sugar. In glucose-sucrose (G-S) medium, the utilization of

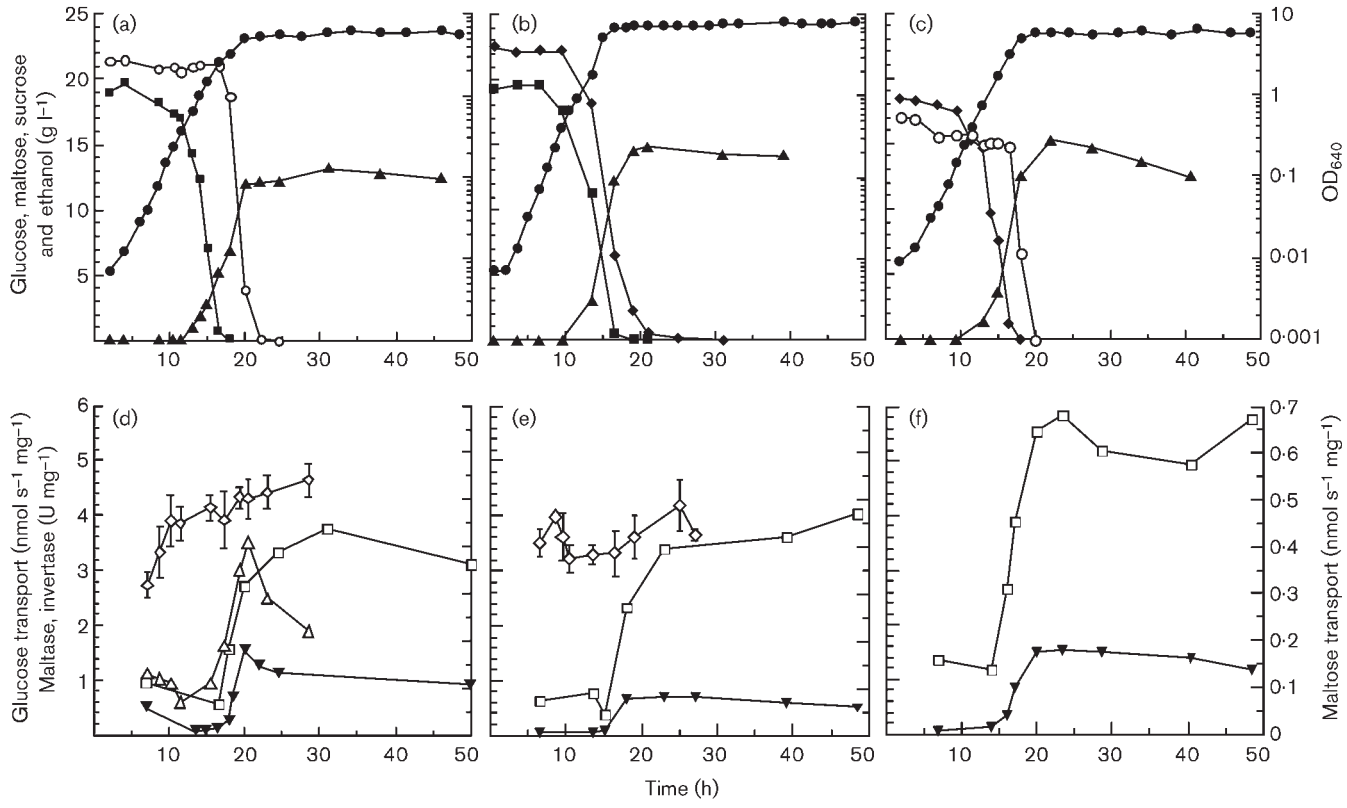

Fig. 1. Growth of *T. delbrueckii* in YP medium containing mixtures of glucose-maltose (a, d), glucose-sucrose (b, e) and sucrose-maltose (c, f). ●, OD_{640}; ■, glucose; ○, maltose; ◆, sucrose; ▲, ethanol; ▼, maltase activity [U (mg protein)$^{-1}$]; □, invertase activity [U (mg protein)$^{-1}$]; ◇, △, rate of glucose (◇) and maltose (△) transport [nmol s^{-1} (mg dry weight)$^{-1}$]. The data are representative of the results obtained in two independent experiments. The experimental variation was below 2 % for all measurements except for glucose transport, where standard deviations are presented.

both sugars was simultaneous (Fig. 1b). However, in S-M medium sucrose was apparently preferred to maltose (Fig. 1c). Following an initial slow consumption of maltose, concomitant with sucrose utilization, a lag period was observed which lasted until sucrose had almost disappeared. Only then was maltose consumption resumed.

Specific growth rates, biomass and ethanol yields were estimated for the growth conditions tested and their values compared for *T. delbrueckii* PYCC 5321 and *S. cerevisiae* PYCC 5325 (Table 1). In single-sugar media, the growth rate values were similar, although slightly higher in glucose and sucrose media than in maltose medium for both yeasts. In mixed-sugar media, the values were similar to those obtained in single-sugar media (Fig. 1, Table 1). As for biomass yields, typical values for fermentative metabolism were obtained in all cases. However, the biomass yields were slightly lower for *S. cerevisiae* than for *T. delbrueckii* (Table 1) in either glucose or maltose medium. Ethanol yields in sugar mixtures were higher when using *S. cerevisiae*, except in S-M medium, where similar values were observed (Table 1). For comparison, the pattern of maltose utilization when in the presence of glucose was also investigated for *S. cerevisiae* PYCC 5325. As expected, sequential sugar consumption was observed. However, maltose started to be consumed when glucose was still detectable in the medium (not shown), suggesting a higher glucose control over maltose metabolism in *T. delbrueckii* than in the commercial baker's yeast.

To characterize further the utilization of mixed sugars in *T. delbrueckii*, the activities of two key enzymes, invertase and maltase, were followed during the fermentations (Fig. 1d–f). For all tested media, maltase and invertase activities remained at low levels while glucose (in G-M and G-S media) or sucrose (in S-M medium) were present and increased concomitantly as these sugars approached depletion. Interestingly, maximal maltase activity of *T. delbrueckii* in G-M medium was comparable to the value obtained with *S. cerevisiae*, although for the latter species maltase activity was detected before glucose was completely consumed (results not shown), a result in accordance with the observed maltose utilization when glucose was still being consumed. Furthermore, while maltase activity in *T. delbrueckii* reached higher values whenever maltose was present in the medium, maximal invertase activity in the absence of sucrose (G-M medium) was similar to that found in glucose-sucrose medium (Fig. 1d–f). However, both enzymes were subject to glucose repression. Similar results were observed upon growth in synthetic medium (data not shown), hence excluding the possible interference of residual amounts of sugars contained in YP-based media.

Sugar transport

To investigate a possible relation between specific sugar consumption rates inferred from the values shown in Table 1 ($q_{sugar} = \mu / Y_x$) and the first step of maltose and glucose metabolism, the transport of these two sugars was evaluated during the fermentations in mixed-sugar media (Fig. 1d, e). Just like *S. cerevisiae*, *T. delbrueckii* is known to transport maltose through a maltose-H^+ symport mechanism, inducible and subject to glucose repression (Alves-Araújo *et al.*, 2004b). Accordingly, in G-M medium we observed that maltose transport capacity increased only after glucose exhaustion (Fig. 1d). The maximum maltose transport capacity obtained [0.41 nmol s^{-1} (mg dry weight)$^{-1}$: Fig. 1d)] was about one and a half times lower than that obtained for cells grown in YP maltose medium [$V_{max} = 0.66$ nmol s^{-1} (mg dry weight)$^{-1}$], which in turn is lower than the estimated specific maltose consumption rate [$q_{maltose} = 1.8 \pm 0.3$ nmol s^{-1} (mg dry weight)$^{-1}$]. This suggests that maltose uptake may be limiting maltose metabolism. A similar analysis was conducted for glucose. Glucose transport in *T. delbrueckii* follows a biphasic Michaelis–Menten kinetics with low- and high-affinity components (Alves-Araújo *et al.*, 2005). During exponential growth in G-M and G-S media, glucose uptake rates were very similar (Fig. 1d, e). The estimated specific glucose consumption rate [$q_{glucose} = 4.1 \pm 0.4$ nmol s^{-1} (mg dry weight)$^{-1}$] in YP glucose medium was comparable to the total capacity of glucose transport [$V_{max} = 3.96 \pm$

Table 1. Specific growth rates, biomass and ethanol yields from batch cultures in YP medium supplemented with different sugars, either alone or in mixtures

μ, specific growth rate (h^{-1}); Y_x, biomass yield [g (g substrate carbon)$^{-1}$]; Y_E, ethanol yield [g (g substrate carbon)$^{-1}$]. Data are means ± SD of at least three independent experiments.

		Carbon source					
		Glucose	Sucrose	Maltose	Glucose-maltose	Glucose-sucrose	Sucrose-maltose
T. delbrueckii	μ	0.56 ± 0.06	0.59 ± 0.04	0.48 ± 0.07	0.50 ± 0.05	0.55 ± 0.03	0.52 ± 0.06
	Y_x	0.53 ± 0.01	0.54 ± 0.05	0.50 ± 0.04	0.37 ± 0.09	0.35 ± 0.03	0.38 ± 0.09
	Y_E	–	–	–	0.86 ± 0.06	0.94 ± 0.08	1.06 ± 0.02
S. cerevisiae	μ	0.67 ± 0.05	0.68 ± 0.04	0.62 ± 0.05	0.65 ± 0.06	0.64 ± 0.04	0.64 ± 0.07
	Y_x	0.48 ± 0.10	0.54 ± 0.10	0.42 ± 0.04	0.31 ± 0.03	0.39 ± 0.05	0.33 ± 0.06
	Y_E	–	–	–	1.08 ± 0.05	1.10 ± 0.10	1.07 ± 0.06

0.56 nmol s^{-1} (mg dry weight)$^{-1}$ in G-M and 3.60 ± 0.32 nmol s^{-1} (mg dry weight)$^{-1}$ in G-S], indicating that glucose metabolism may well be limited by glucose transport.

Sugar metabolism

Respiratory and fermentative capacities of *T. delbrueckii* PYCC 5321 and *S. cerevisiae* PYCC 5325 cells grown in YP with glucose, maltose or sucrose as the only carbon and energy sources were determined using the Warburg method. The results, expressed as specific CO_2 production (qCO_2) and oxygen consumption (qO_2) rates, are presented in Table 2. The data obtained with *T. delbrueckii* show that all sugars tested are essentially fermented (77–88 % of the total sugar supplied) and that the fermentation rates were higher for sucrose and glucose than for maltose (Table 2), which is in accordance with the lower values obtained for $q_{maltose}$ and $\mu_{maltose}$ (Table 1). It is noteworthy that the qCO_2 values for sucrose and glucose were always similar, irrespective of the sugar used for growth (Table 2). However, fermentation rates obtained with maltose were significantly higher ($P<0.001$) in maltose-grown cells than in either sucrose- or glucose-grown cells (Table 2).

S. cerevisiae PYCC 5325 displayed a somewhat different behaviour. The fraction of glucose fermented (approx. 96–97 %) was higher than in the case of sucrose or maltose (around 93 %), as readily inferred from the RQ values (Table 2). No significant differences between the fermentation rates of maltose were observed in cells grown with any of the three sugars (Table 2), in contrast to the values obtained for glucose and sucrose fermentation. In both cases, the qCO_2 values were found to be higher in glucose- or sucrose-grown cells and lower in maltose-grown cells ($P<0.001$ and $P<0.01$, respectively).

A comparative analysis between *T. delbrueckii* and *S. cerevisiae* regarding the specific oxygen consumption rates (qO_2) estimated with the different sugars showed similar values for sucrose, whereas the values almost doubled for *T. delbrueckii* in the case of glucose and maltose (Table 2). As stressed above, the relative contribution of respiration to sugar catabolism is always higher in *T. delbrueckii*, the RQs varying between 3.43 (sucrose) and 2.12 (maltose). For *S. cerevisiae*, RQ values were in the range 5.41–9.70, which reflects the higher fermentative capacity of this yeast.

DISCUSSION

T. delbrueckii is nowadays an important case study among the non-*Saccharomyces* yeast species, with particular relevance to the baking and wine industries. Among the most important characteristics of a good baker's yeast is the dough-leavening ability, which implies the efficient fermentation of both maltose and glucose, and high biomass productivities on sucrose, the major sugar in molasses used as raw material to produce the yeast. Although the patterns of sugar utilization by *T. delbrueckii* are very similar to those described for *S. cerevisiae* (Mormeneo & Sentandreu, 1982; Needleman, 1991; Gancedo, 1998), a few significant differences were observed. In G-M medium the pattern was almost identical, the increase in maltose transport and maltase activities clearly coinciding with the outset of maltose consumption. Hence, there is an apparent co-regulation of these proteins, both being subject to glucose repression and induction by maltose. The results obtained are consistent with the previous identification of a bifunctional *MAL* promoter in *T. delbrueckii* PYCC 5321, shared by maltase and maltose transporter genes, including Mig1p and UAS_{MAL} consensus binding sites (Alves-Araújo *et al.*, 2004b). The glucose control over maltose metabolism was stricter in *T. delbrueckii* PYCC 5321, since *S. cerevisiae*

Table 2. Specific fermentation and respiration rates of *T. delbrueckii* and *S. cerevisiae* grown in media with different sugars

Yeasts were grown in YP medium supplemented with 20 g l^{-1} of the indicated sugar, and harvested at the exponential phase (OD_{640} 0.8–0.9). CO_2 production and O_2 consumption rates are expressed as mmol h^{-1} (g dry weight)$^{-1}$. RQ = qCO_2 (total)/qO_2. Values are means ± SD of three independent experiments.

Sugar in medium	Sugar in assays	*T. delbrueckii* PYCC 5321			*S. cerevisiae* PYCC 5325		
		qCO_2	qO_2	RQ	qCO_2	qO_2	RQ
Glucose	Glucose	6.36 ± 0.43	2.92 ± 0.70	3.18 ± 0.79	14.26 ± 0.71	1.64 ± 0.44	9.70 ± 2.6
	Sucrose	6.42 ± 0.85	–	–	14.22 ± 0.52	–	–
	Maltose	2.79 ± 0.22	–	–	11.54 ± 1.17	–	–
Sucrose	Glucose	5.57 ± 1.26	–	–	12.97 ± 0.63	–	–
	Sucrose	6.94 ± 0.54	2.86 ± 0.26	3.43 ± 0.41	12.29 ± 0.81	2.79 ± 0.19	5.41 ± 0.51
	Maltose	2.74 ± 0.09	–	–	10.08 ± 1.05	–	–
Maltose	Glucose	5.65 ± 0.92	–	–	10.13 ± 0.44	–	–
	Sucrose	6.07 ± 0.91	–	–	10.50 ± 1.26	–	–
	Maltose	5.59 ± 0.50	5.01 ± 0.61	2.12 ± 0.32	12.45 ± 1.20	2.77 ± 0.12	5.50 ± 0.58

PYCC 5325 started to consume maltose when glucose was still detectable in the medium. In glucose-maltose mixtures, under laboratory culture conditions, this differential behaviour of the two species could lead to an undesirable delay in CO_2 production from maltose by *T. delbrueckii*. However, this advantage exhibited by *S. cerevisiae* is counteracted under the conditions prevailing in bread dough by the higher osmotolerance of *T. delbrueckii*. Indeed, the gas production capacity of *T. delbrueckii* PYCC 5321 in lean dough was slightly lower than the leavening capacity of *S. cerevisiae* PYCC 5325 (Almeida & Pais, 1996b) and slightly higher than the rates obtained with other commercial baker's yeast strains (Hernandez-Lopez *et al.*, 2003). The differences reported by the latter authors were even more pronounced in sweet, sucrose-added, doughs. In sucrose medium, *T. delbrueckii* PYCC 5321 showed a lower growth rate than *S. cerevisiae* PYCC 5325, although the biomass yields were equivalent. This is consistent with the higher contribution of respiration to the overall sugar metabolism in *T. delbrueckii*. Since the biomass productivity, in industrial fed-batch cultures, is limited not only by the substrate concentration but also by the oxygen available, the growth potential of *S. cerevisiae* is countered by its requirement for a more careful monitoring of the oxygen tension, to prevent ethanol production. The level of invertase activity in rich YP medium is similar for both yeasts and the regulatory mechanisms for this enzyme appear to be the same. A correlation between invertase activity and sucrose consumption is unclear and needs further investigation.

Overall, the experimental evidence points to sugar transfer rates into the cell limiting the efficiency of the fermentation. In the case of sugar mixtures with maltose, the inhibitory effect of maltose on glucose uptake, which is known to occur in *S. cerevisiae* (Diderich *et al.*, 1999) and was also found in *T. delbrueckii* (Alves-Araújo *et al.*, 2005), could reinforce this limitation. In particular, in S-M mixtures (Fig. 1c) the maltose concentration surpasses by far the glucose resulting from extracellular sucrose hydrolysis, thus possibly hindering glucose (and fructose) utilization. This would mean that, in the absence of maltose, sucrose could be consumed faster, which in reality was not observed. On the contrary, it seems that the glucose being released from sucrose through the action of the invertase inhibits maltose metabolism through mechanisms of glucose repression. More detailed studies are required to evaluate these aspects.

Despite the clear fermentative metabolism of *T. delbrueckii*, with the production of high ethanol yields in batch cultures with each of the sugars tested, our data on the specific rates of CO_2 production and O_2 consumption, estimated with the Warburg manometric technique, showed a higher contribution of respiration in *T. delbrueckii* compared to *S. cerevisiae*. It is worth noting that during batch cultivation the available oxygen rapidly reaches limiting concentrations, thereby favouring fermentative metabolism. In fact, when biomass yields were determined in YP medium, with either glucose, sucrose or maltose, using higher aeration rates a very significant increase in biomass yields (from 20 %, in glucose or sucrose medium, to 80 %, in maltose medium) was observed (not shown). As emphasized above, a more efficient modulation of the respiratory metabolism in *T. delbrueckii* under aerobic conditions represents an asset for the large-scale production of yeast.

As a final remark, the strain *T. delbrueckii* PYCC 5321 used in the present work was reported to display a much higher freezing and osmotic tolerance than *S. cerevisiae* (Almeida & Pais, 1996a, b; Alves-Araújo *et al.*, 2004a), properties of special interest for the baking industry. In addition to these characteristics, our results show that *T. delbruecki* behaves very similarly to *S. cerevisiae* with respect to sugar utilization and regulation patterns. This work also indicated that maltose uptake is a good target for metabolic engineering and improvement of *T. delbrueckii*'s performance in bread doughs. The present study further contributes to the characterization of *T. delbrueckii* PYCC 5321 at the physiological and biochemical levels, bridging a gap for its exploitation by the baking industry and increasing knowledge on the so-called non-conventional yeast species.

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